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The Role of Polymorphic Protein Tyrosine Phosphatase Non-Receptor Type 22 in Leprosy

Journal of Investigative Dermatology (2009) **129,** 2726–2728; doi:10.1038/jid.2009.140; published online 11 June 2009

TO THE EDITOR

Leprosy, caused by *Mycobacterium leprae*, presents a spectrum of findings, with tuberculoid leprosy at one end and lepromatous leprosy at the other. Although humoral immune responses are observed throughout the spectrum, cell-mediated immunity is observed in the tuberculoid leprosy form of the disease (Ridley and Jopling, 1966). We previously showed a much stronger association of the *HLA* allele, *DRB1* 1501*, with multibacillary leprosy than with tuberculoid leprosy and a significant decrease of *DRB1*0701* in multibacillary leprosy as compared with the tuberculoid form and controls (Rani *et al.*, 1993). This suggested that *HLA* alleles may play a role in differentiating the manifestations of leprosy. However, an integrated role for genes involved in immune responses cannot be ruled out. Given that the T cells are anergic to *M. leprae* antigens in leprosy patients, we explored the role of protein tyrosine phosphatase non-receptor type 22 (*PTPN22*) in that disease.

PTPN22 encodes for an 807 amino acid residue protein called LYP (lymphoid tyrosine phosphatase), which has been shown to negatively regulate T-cell signaling (Hasegawa *et al.*, 2004). A single-nucleotide polymorphism in the *PTPN22* gene at nucleotide position 1858 C>T (codon 620), resulting in an arginine-to-tryptophan (CGG to TGG) transition, has been shown to be a gain-of-function mutation, with a more potent negative regulation of T-cell signaling through reduced Lck (leukocyte-specific protein tyrosine kinase)-mediated phosphorylation of the TCRξ chain, reduced tyrosine phosphorylation of LAT (linker for activation of T cells), and reduced activation of Erk2 (Vang *et al.*, 2005). The mutant, LYP-Trp620, has been associated with several autoimmune diseases (Begovich *et al.*, 2004; Bottini *et al.*, 2004; Kyogoku *et al.*, 2004) Recently, Chapman *et al.* (2006) have shown its involvement in invasive pneumococcal disease and Gram-positive bacterial disease. Owing to its involvement in the downregulation of T-cell function and in invasive bacterial disease, one could hypothesize that LYP-Trp620 has a role in the manifestation of mycobacterial diseases as well.

To study the role of the *PTPN22* C1858T single-nucleotide polymorphism, 153 leprosy patients from North India—103 lepromatous patients, including borderline lepromatous patients (79 men and 24 women with a mean (±SD) age of 32.26±11.84 years), and 50 tuberculoid patients, including borderline tuberculoid patients (38 men and 12 women with a mean (±SD) age of 31.35±9.16 years)—diagnosed on the basis of immunological, histopathological, and bacteriological status, were compared with 365 ethnically matched healthy controls (191 men and 174 women with a mean (±SD) age of 36.02±10.31 years), using PCR followed by restriction digestion with the enzyme *Rsa*1, as described by Zheng and She (2005). The study was approved by the Human Ethics Committees of Ram Manohar Lohia Hospital and National Institute of Immunology. The restriction endonuclease, *Rsa*1, cleaves the DNA strand that contains the C nucleotide at the 1858th position and exhibits two bands of 176 and 42 bp. The mutant, 1858T, however, is not digested by *Rsa*1 and shows a single band of 218 bp (Supplementary Figure 1). All samples showing CT heterozygosity were repeated for both amplification and restriction digestion by another individual to confirm the genotyping. A total of 20% of the CC homozygous samples were repeated for both amplification and restriction digestion randomly, and the restriction digestion clearly showed the 176 bp band.

The frequency of the 1858T allele was significantly higher in both lepromatous ($P<0.00006$) and tuberculoid ($P<0.001$) leprosy patients than in normal healthy controls (Table 1). Although homozygous 1858TT was absent from both groups of patients, as well as from the control samples studied, heterozygous CT was significantly increased in both groups of patients (Table 1). All genotype frequencies in patients as well as in controls were in Hardy–Weinberg equilibrium. The allele frequency of the

Abbreviations: PTPN22, protein tyrosine phosphatase non-receptor type 22

Table 1. Allele and genotype frequencies of PTPN22 C1858T and predisposing HLA alleles in leprosy patients as compared with normal healthy controls

Allele/ genotype	Lepromatous leprosy[1]		Tuberculoid leprosy[1]		Controls		Lepromatous vs controls		Tuberculoid vs controls		Lepromatous vs tuberculoid	
	No.	%	No.	%	No.	%	*P*	OR[2] (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)
	N=103		*N*=50		*N*=365							
PTPN22												
1858C	190	92.2	92	92	716	98.1	0.00006	0.23 (0.1–0.5)	0.001	0.2 (0.08–0.6)	0.87	1.0 (0.4–2.7)
1858T	16	7.8	8	8	14	1.9	0.00006	4.31 (1.95–9.5)	0.001	4.44 (1.66–11.69)	0.87	0.97 (0.37–2.6)
Genotype CC	87	84.5	42	84	351	96.2	0.00005	0.22 (0.09–0.5)	0.001	0.2 (0.07–0.6)	0.87	1.0 (0.4–2.8)
Genotype CT	16	15.5	8	16	14	3.8	0.00005	4.6 (2.0–10.4)	0.001	4.78 (1.72–13.1)	0.87	0.97 (0.35–2.7)
	N=103		*N*=50		*N*=172							
HLA-DRB1												
DRB1*1501	57	55.3	18	36	36	20.9	$<10^{-6}$	4.7 (2.65–8.3)	0.04	2.12 (1.0–4.4)	0.04	2.2 (1.0–4.7)
DRB1*1502	24	23.3	12	24	20	11.6	0.01	2.3 (1.2–4.7)	0.04	2.4 (1.0–5.7)	0.91	0.96 (0.4–2.3)
DRB1*0701	8	7.8	16	32	59	34.3	1×10^{-6}	0.16 (0.1–0.37)	0.89	0.9 (0.44–1.86)	0.0002	0.2 (0.06–0.5)
DRB1–PTPN22												
1501-CC	49	47.6	16	32	34	19.8	3×10^{-6}	3.6 (2.1–6.6)	0.1	1.9 (0.89–4.1)	0.09	1.9 (0.9–4.2)
1501-CT	8	7.8	2	4	2	1.2	0.006	6.07 (2.1–19.1)	0.22	3.5 (0.86–14.3)	0.31	1.72 (0.6–5.5)
1502-CC	21	20.4	9	18	19	11.1	0.05	2.1 (0.99–4.3)	0.29	1.76 (0.7–4.51)	0.9	1.2 (0.5–3.04)
1502-CT	3	2.9	3	6	1	0.6	0.15	3.98 (0.9–17.8	0.03	8.42 (1.9–30.8)	0.3	0.5 (0.14–1.5)
0701-CC	6	5.8	15	30	56	32.6	1×10^{-6}	0.13 (0.04–0.33)	0.87	0.89 (0.42–1.85)	0.0001	0.14 (0.04–0.44)
0701-CT	2	1.9	1	2	3	1.7	0.62	1.19 (0.3–4.2)	0.64	1.46 (0.01–6.44)	0.62	0.81 (0.16–45.7)

95% CI, 95% confidence interval; HLA, human leukocyte antigen; PTPN22, polymorphic protein tyrosine phosphatase non-receptor, type 22; OR, odds ratio.
[1]Lepromatous leprosy patients include polar lepromatous lepromatous and borderline lepromatous leprosy patients. Tuberculoid leprosy patients include polar tuberculoid and borderline tuberculoid patients.
[2]*P*-values and Odds ratios were calculated using χ^2 using Epistat software, wherever the numbers were less than 5, Fisher's exact test was used to calculate *P*-values and Haldane's modification was used to calculate the Odds ratio in such cases.

minor T allele varies in different ethnic groups. Contrary to reports of an absence of the T allele in Asia (Kawasaki *et al.*, 2006), we observed a very low frequency (1.9%) of this allele in normal healthy individuals from North India as compared with that reported for the populations of European ancestry (ranging from 8.3 to 17%) (Begovich *et al.*, 2004; Kyogoku *et al.*, 2004; Chapman *et al.*, 2006). Earlier reports of an absence of the 1858T allele in individuals from Asia have been from studies conducted in Japan, Korea, and China; these populations are Mongoloid in origin and differ ethnically from Indians. North Indians have been described as being basically Caucasoids, with a racial admixture of Mongoloid and Negroid elements (Rani *et al.*, 1995).

To assess the role of predisposing *HLA* alleles in combination with the *PTPN22* alleles, we studied *HLA-DRB1* alleles in leprosy patients ($N=153$) and controls ($N=172$), using a Labtype SSO kit from One Lambda (Canoga Park, CA), following the manufacturer's instructions, and a Luminex 2.2 flow cytometer (Luminex, Austin, TX). *DRB1*1501*, along with both *PTPN22* 1858CC and CT, was increased significantly in lepromatous patients. There was no significant difference between tuberculoid patients and controls with respect to the simultaneous presence of *DRB1*1501* and *PTPN22* 1858 genotypes (Table 1). However, a significantly higher number of tuberculoid patients had predisposing *DRB1*1501* ($P<0.04$) and *PTPN22* 1858CT ($P<0.001$) independent of each other. Interestingly, the protective *DRB1*0701*, along with *PTPN22* 1858CC, was significantly lower in lepromatous patients as compared with controls ($P<1\times10^{-6}$) and tuberculoid patients ($P<0.0001$). In spite of a significant increase of *PTPN22* 1858CT in tuberculoid patients as compared with controls, a significant increase of *HLA DRB1*0701* along with *PTPN22* 1858CC (both protective), as compared with lepromatous patients who have increased frequencies of both *PTPN22* 1858CC and *PTPN22* 1858CT along with *DRB1*1501*, suggests that these genes have an integrated role in

the manifestations of different forms of leprosy through their functional roles in antigen presentation and inhibition of T-cell responses.

Although predisposing major histocompatibility complex alleles may exhibit inefficient antigen presentation, the LYP-Trp620 allele may have a pathogenic role in the hyporesponsiveness of T cells owing to anomalies in early T-cell signaling, resulting in clinical manifestations of leprosy. Contrary to our expectations, a significantly higher number of tuberculoid patients had *PTPN22* 1858CT, suggesting that there may be early T-cell defects in these patients. This results in a compromised immune response to the infectious agent, which manifests in the milder form of the disease. Most healthy people exposed to *M. leprae* are resistant to the infection, and with an effective immune response they do not develop the disease (Bongiorno *et al.*, 2008). Because the CT genotype accounts for only 15–16% of lepromatous and tuberculoid patients, other genes involved in the downregulation of T-cell responses, such as *CTLA-4* and *Foxp3*, should be investigated for additional host factors that may be detrimental in conferring anergy to *M. leprae* antigens in lepromatous leprosy patients.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

The authors are thankful to Ravinder Goswami, AIIMS, for control data from 193 healthy individuals. The technical assistance of Kapoor Chand is gratefully acknowledged. The funds for this study came from a National Institute of Immunology (NII) core grant. The authors acknowledge Chandrima Shaha, NII, for suggestions.

Rajni Rani[1], Anamika Singh[1], Neetu Israni[1], Archana Singh[1], Pankaj Sharma[2] and Hemant K. Kar[2]

[1]Molecular Immunogenetics Group, National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi, India and [2]Department of Dermatology, Dr Ram Manohar Lohia Hospital, New Delhi, India
E-mail: rajni@nii.res.in

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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IgA Anti-Epidermal Transglutaminase Antibodies in Dermatitis Herpetiformis and Pediatric Celiac Disease

Journal of Investigative Dermatology (2009) **129,** 2728–2730; doi:10.1038/jid.2009.142; published online 11 June 2009

TO THE EDITOR

Recent studies have suggested epidermal transglutaminase (eTG) as the autoantigen of dermatitis herpetiformis (DH) (Sardy *et al.*, 2002) (Donaldson *et al.*, 2007), but the patient cohorts in published studies on its clinical utility in DH have been small. IgA anti-eTG is present in about 50% of adult celiac disease (CD) (Hull *et al.*, 2008); however, its prevalence in a large cohort of pediatric CD has not been reported. The primary objective of this study was to confirm and expand on our previously published data, and to determine the clinical utility of IgA anti-eTG in a large cohort of DH patients. Our second objective was to further evaluate the relative prevalence of IgA anti-eTG in a larger cohort of pediatric CD patients.

Abbreviations: Ab, antibody; CD, celiac disease; DH, dermatitis herpetiformis; eTG, epidermal transglutaminase; tTG, tissue transglutaminase